Thermogenesis and Thermoregulatory Function of Iron-Deficient Women Without Anemia

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Physiological responses at 16°C were studied in 11 women, age 28 ± 2 (mean ± S.E.) years and 26 ± 2% fat, after their body iron stores were depleted by diet (5.0 mg iron · 2,000 kcal^{-1} · d^{-1}), phlebotomy and menstruation for about 80 d and were repleted by diet (13.7 mg iron · 2,000 kcal^{-1} · d^{-1}) for about 100 d, including daily iron supplementation (50 mg of iron as ferrous sulfate) for the last 14 d of repletion. Iron depletion was characterized by a decline ($p < 0.05$) in hemoglobin (12.0 ± 0.2 g · dl^{-1}), ferritin (5.5 ± 0.5 ng · ml^{-1}) and body iron balance (−9.1 ± 2.6 mg · 6 d^{-1}). Iron repletion, including supplementation, increased ($p < 0.05$) hemoglobin (12.6 ± 0.1 g · dl^{-1}), ferritin (9.5 ± 0.4 ng · ml^{-1}) and iron balance (+67 ± 6.7 mg · 6 d^{-1}). Iron depletion reduced ($p < 0.05$) metabolic heat production (49.6 ± 1.1 vs 53.6 ± 1.2 W · m^{-2}) during acute cold exposure. The rates of cooling of the core and periphery were greater ($p < 0.05$) during iron depletion than repletion. A shift in the lower core temperature threshold for shivering was paralleled by an earlier onset of shivering ($p < 0.05$) in iron depletion indicating an adaptation in cold tolerance in an attempt to maintain core temperature. Iron depletion was associated with blunted post-exposure increases in plasma thyroid hormone concentrations and greater ($p < 0.05$) increases in plasma norepinephrine concentrations as compared to iron repletion. In a subsample of the women, no significant effect of calcium or ascorbic acid supplementation was found on responses to cold exposure. These findings indicate that iron deficiency is associated with impaired thermogenesis and an adaptive thermoregulatory response during acute cold exposure.

IRON DEFICIENCY is a leading nutritional problem throughout the world. It is estimated to affect 10–15% of the women aged 12–45 years in the United States (28). Iron deficiency is not a single entity; rather, it represents a spectrum of biochemical, and potentially physiological, characteristics. It has been proposed (8) that iron deficiency develops in three stages. Initially, stored iron pools in tissues and organs are reduced. A brief and transient period follows in which circulating iron concentrations and transport iron decline. Finally, erythropoiesis decreases markedly, hemoglobin production declines, and anemia occurs.

Investigators have described the deleterious effects of iron deficiency on physiological function, including work capacity and temperature regulation in the cold, by studying anemic individuals (24,31). Although reduced oxygen carrying capacity is assumed to be the greatest contributor to impaired physiological function in anemia, there is evidence that iron deficiency without anemia depletes cellular enzymes (14).

When exposed to an acute cold stressor, iron-deficient rats demonstrated a greater decline in rectal temperature than did control animals (10). These iron-deficient rats showed a disproportionate increase in circulating norepinephrine (10) and an impairment in the conversion of thyroxine (T_4) to triiodothyronine (T_3) (11).

Preliminary reports suggest that nutritional factors other than iron may also play a role in regulating body temperature in the cold. An early study (12) found that ascorbic acid supplementation of monkeys acclimatized to moderate cold reduced the fall in core and muscle temperatures when the animals were exposed to severe cold. Studies (13,27) of direct injection of ionic calcium into the hypothalamus of unanesthetized cats showed that calcium and sodium are important in maintaining the body temperature set point.

The present study was undertaken to determine whether a decline in the ability to maintain body temperature also occurs in iron-deficient but not anemic humans exposed to a cold environment. A second goal was to investigate whether supplemental calcium and ascorbic acid influence human physiological responses during acute cold exposure.

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MATERIALS AND METHODS

Subjects: Eleven healthy women, aged 22–36, with normal serum ferritin and hemoglobin concentrations were recruited. The volunteers entered the study after they had been informed in detail of the nature of the research, including the risks and benefits, and after medical, nutritional, and psychological evaluations established that they had no underlying disease and were emotionally suited for this project. This investigation was approved by the Human Studies Review Boards of the United States Department of Agriculture and the University of North Dakota School of Medicine, and followed the guidelines of the Department of Health and Human Services and the Declaration of Helsinki regarding the use of human subjects.

Experimental protocol: The women lived in a metabolic unit under close supervision for 180 d. The volunteers consumed only food and drink provided by the staff of the metabolic unit and collected all excreta as described elsewhere (21,25). When outside the metabolic unit, the women were chaperoned by trained staff members. Physical activities on and off the unit were monitored and controlled using a standardized method of prescription of exercise and activity to maintain work capacity and body composition at entry levels (22). They were depleted of body iron with a diet low in iron and with blood loss as a result of phlebotomy and menstruation until serum ferritin concentrations declined to less than 8.5 $ng \cdot ml^{-1}$. The iron depletion period required about 80 d and, because of individual responses, it ranged from 67 to 88 d. Women who became iron depleted more rapidly began an iron repletion diet and their iron status was maintained by continued phlebotomy, which was reduced to minimal amounts after 88 d. Six of the women received 800 mg supplemental calcium daily during iron depletion to determine the influence of calcium on iron absorption (9) and temperature regulation in the cold. The calcium supplements did not affect iron balance or indices of iron status (17).

During iron repletion, the diets were supplemented with either placebo or ascorbic acid, 500 mg three times daily (1,500 mg total) with major meals, to examine the effect of ascorbic acid on iron absorption (17) and physiological responses during acute cold exposrue. Five volunteers received the ascorbic acid supplements for about 100 d.

Diet: The participants were fed constant weight diets on a 3-d menu rotation. The diets were prepared using conventional western foods. The iron depletion diet contained 5.0 mg iron • 2,000 $kcal^{-1}$. The average daily iron intake was 6.1 mg, and it ranged from 4.7 to 7.6 $mg \cdot d^{-1}$. The unsupplemented diet contained 806 mg of calcium and 59 mg ascorbic acid • 2,000 $kcal^{-1}$. The average daily intake of calcium was 1,080 $mg \cdot d^{-1}$ (range = 645–1,510 $mg \cdot d^{-1}$) and of ascorbic acid was 72 $mg \cdot d^{-1}$ (range = 48–95 $mg \cdot d^{-1}$).

The iron repletion diet provided 13.7 mg iron • 2,000 $kcal^{-1}$. The average iron intake was 17.4 $mg \cdot d^{-1}$. The additional iron sources in this diet were from vegetables. A daily 50 mg iron supplement as ferrous sulfate was added during the last 14 d of the repletion phase. The energy distribution in these diets was about 51% as carbohydrate, 11–13% as protein, and 36–39% as fat.

Analyses: Diets, excreta, and menstrual fluid losses were collected continuously throughout the study with precautions to avoid trace element contamination. Duplicate diets were prepared for analyses throughout the study. Six-day composites of diets and feces were prepared by homogenization in 1 gal stainless steel blenders. The feces were frozen, then lyophilized before blending. Aliquots of the dietary and fecal composites were digested with concentrated nitric and 70% perchloric acids by method (II)A of the Analytical Methods Committee (2). The iron content of the digestates was determined using inductively-coupled plasma emission spectrophotometry with aqueous calibration standards. Urinary iron was measured by atomic absorption spectrophotometry (25). Iron balance was expressed as $mg \cdot 6\ d^{-1}$ balance period to reduce intraindividual variability in calculated iron balance caused by variable stool output.

To estimate menstrual losses of iron, pads, tampons, and gauze wipes, selected for low trace element contamination, were collected in plastic bags daily during menstruation. Care was taken to avoid trace metal contamination. The menstrual collection materials were extracted with 0.001 M hydrochloric acid. An aliquot was taken for hemoglobin analysis. Iron was determined using additional aliquots of the extract after digestion with nitric acid and hydrogen peroxide. Unused collection materials were taken through the same procedure to correct for background levels of iron.

Iron balance was calculated as the difference between iron intake and losses due to extraction, phlebotomy, and menstruation. Iron loss through phlebotomy was estimated as (26):

$$[3.4\ (Hgb) + Fe\ (1\text{-}Hct \times 10^{-2})]\ [10^{-2}] \times \text{blood loss}$$

where 3.4 is the factor used to estimate the iron concentration of each gram of hemoglobin (e.g., 3.4 mg iron • 1 g $hemoglobin^{-1}$); Hgb is hemoglobin concentration ($g \cdot dl^{-1}$); Fe is plasma iron concentration ($mg \cdot dl^{-1}$); Hct is hematocrit (%); blood loss is in milliliters.

Fasting venous blood samples were taken at regular intervals for the determination of biochemical indices related to iron nutriture. Hemoglobin and hematocrit were determined using a standard method and instrumentation (Coulter Model S, Coulter Electronics, Hialeah, FL[1]). Serum ferritin concentrations were determined using a commercial radioimmunoassay (Clinical Assays, Cambride, MA).

Body composition: Assessment of body composition was performed using hydrodensitometry and the method and procedures described by Akers and Buskirk (1). Percent body fat was calculated from body density using the equation of Brozek, *et al.* (5). Skinfold thicknesses were measured using a calibrated skinfold caliper at five sites (biceps, triceps, subscapular, suprailiac, and anterior mid-thigh) on the right side of the body.

Cold exposure: The acute cold stressor tests were

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

conducted in an environmental chamber at 16°C and 50% relative humidity with an air flow of less than 15 $m \cdot min^{-1}$. All tests were scheduled at the end of the iron depletion and the iron repletion and supplementation periods and were performed in the morning after an overnight fast. Because core temperature is dependent upon phases of the menstrual cycle (30), cold exposures were performed on days 5–10 of the follicular phase. After voiding her bladder, each volunteer put on a two-piece swim suit before an indwelling teflon catheter was inserted into an antecubital vein. After a 45 min rest in a supine position at room temperature (23–24°C), a blood sample was obtained for hormone analyses.

During cold exposure, oxygen consumption and carbon dioxide production were determined by indirect calorimetry with an automated system (Sensormedics, Anaheim, CA) using a tightly-fitting face mask. Oxygen and carbon dioxide analyzers were calibrated before each test with reference gas mixtures whose compositions were previously determined by standard chemical procedures. The oxygen and carbon dioxide concentrations in the expired air were determined over 2-min periods. Rectal or core temperature was measured using a calibrated thermistor (YSI Series 700; Yellow Springs, OH). Skin temperatures were determined at nine sites (finger, forearm, upper arm, forehead, chest, abdomen, thigh, lower leg, and toe) using calibrated thermocouples placed on the right side of the body. Data were recorded every minute using a computerized data acquisition sysem (Series 500, Keithley Instruments, Inc., Boston, MA).

Using these primary data, other physiological variables including energy production (M), weighted mean skin temperature ($\bar{T}_{sk}$), mean body temperature ($\bar{T}_b$, and body heat debt (S) were calculated according to the following formulae (6):

$$M\ (W^{-1}m^{-2}) = [(CE)\ (\dot{V}o_2)(1.163)]/(SA)$$

where $\dot{V}o_2$ is liters of oxygen consumed per hour; CE is the non-protein caloric equivalent of 1 L of oxygen based upon the measured respiratory exchange ratio (23); 1.163 is a conversion factor from $kcal \cdot h^{-1}$ to watts (W); and SA is surface area in m^2.

$$\bar{T}_{sk}\ (°C) = 0.20\ T_{forehead} + 0.30\ T_{chest} + 0.15\ T_{upper\ arm} + 0.05\ T_{forearm} + 0.05\ T_{finger} + 0.15\ T_{thigh} + 0.05\ T_{calf} + 0.05\ T_{toe}$$

where T is temperature (°C).

$$\bar{T}_b\ (°C) = 0.67\ T_{re} + 0.33\ \bar{T}_{sk}$$

where T_{re} is rectal temperature (°C).

$$S\ (W) + [0.83\ Wt\ (\bar{T}_{b1} - \bar{T}_{b2})(1.163)/(t_1 - t_2)$$

where 0.83 is the assumed average specific heat of the body, $kcal \cdot kg \cdot °C^{-1}$; Wt is body weight in kg; and $\bar{T}_{b1} - \bar{T}_{b2}$ is the change in mean body temperature (°C) from time 1 to time 2; 1.163 is a conversion factor from $kcal \cdot h^{-1}$ to W; and $t_1 - t_2$ is duration of time from time 1 to time 2 in minutes.

Each cold exposure was terminated at the onset of visible shivering. Shivering was assessed by one test supervisor (CBH) who observed the activity of three skeletal muscle groups (sternocleidomastoidal, pectoral, and abdominal muscles) using a remote control camera with a close-up lens during the last 15 s of each minute of cold exposure. When involuntary contraction of any of these muscle groups was observed for two consecutive time periods (e.g., 2 min), the volunteer was designated to be at the onset of visible shivering and then was removed from the cold.

A venous blood sample also was obtained at the end of each cold stressor test.

Pre- and post-exposure plasma samples were analyzed for catecholamines using high pressure liquid chromatography with electrochemical detection (15). Serum free T_4 (Baxter Clinical Assays, Cambridge, MA), free T_3 (Dade, Cambridge, MA), and thyroid-stimulating hormone (TSH, Dade, Cambridge, MA) concentrations were determined using commercial radioimmunoassay kits.

Statistics: The values are presented as the mean ± S.E. Statistical comparisons of biochemical, nutritional, and physiological variables by experimental treatment were performed using the analysis of variance with repeated measures design (19) with a Scheffé post hoc test (29) as were comparisons of the effects of dietary supplements on physiological responses. Linear regression analysis was used to describe physiologic responses over time during cold exposure. Comparisons of slopes and intercepts of regression lines were performed using the analysis of variance (20).

RESULTS

The physical characteristics of the women are shown in Table I. There was no effect ($p > 0.05$) of dietary iron on body weight or body composition. Iron balance during the last two 6-d balance periods of depletion was −9.1 ± 2.6 mg which was less ($p < 0.05$) than iron balance at the end of repletion (+28.9 ± 5.1 mg). Re-

TABLE I. CHARACTERISTICS OF 11 FEMALE VOLUNTEERS*

	Entry	Depletion	Repletion and Supplementation
Age, yr	28 ± 2	—	—
Stature, cm	167.6 ± 2.9	—	—
Body mass, kg	67.1 ± 4.9	66.9 ± 4.8	66.6 ± 4.8
Sum of five skinfolds†, mm	71.3 ± 7.9	72.5 ± 7.7	71.8 ± 7.6
Body fat††, %	28.0 ± 1.5	28.7 ± 1.3	28.0 ± 1.3
Fat-free mass, kg	47.6 ± 4.6	47.4 ± 4.4	47.5 ± 4.7

* Values are mean ± S.E.
† Biceps, triceps, subscapula, suprailiac, and anterior mid-thigh.
†† Estimated by densitometry (1) and calculated according to Brozek, *et al.* (5).

pletion plus iron supplementation resulted in an even greater ($p < 0.05$) iron retention ($+67 \pm 6.7$ mg) which is partially the result of a delay in eliminating some of the ingested iron that was not absorbed.

Some changes in blood biochemical indices were observed (Table II). Mean hemoglobin and hematocrit declined ($p < 0.05$) during iron depletion. While the group average hemoglobin was 12.0 $g \cdot dl^{-1}$, a value not indicative of anemia (7), four of the women had values ranging from 11.6 to 11.9 $g \cdot dl^{-1}$. Ferritin also decreased ($p < 0.05$) following depletion. With iron repletion and supplementation, hemoglobin and hematocrit returned to entry values. Serum ferritin was greater ($p < 0.05$) after repletion and supplementation than after depletion, but did not return to admission values.

The onset of visible shivering was observed earlier ($p < 0.05$) during iron deficiency (83 ± 2 min; range = 76–86 min) than after iron repletion and supplementation (94 ± 3 min; range = 90–98 min). Subjectively, each subject expressed discomfort after the initial minutes in the cold. This feeling seldom passed away completely and remained throughout the course of cold exposure. The observation of the onset of visible shivering was in agreement with the perceptions of the volunteers. Inter-individual variability in the onset and the extent of shivering after each dietary period was moderate, probably because body fatness among the women was similar and the general nutritional and biochemical responses to experimental treatments were consistent.

Average core and mean skin temperature declined during cold exposures (Fig. 1). There was no significant difference between either core temperatures or mean skin temperatures at room temperature relative to iron status. Iron depletion was associated with depressed ($p < 0.05$) core and mean skin temperatures during cold exposure. The rate of cooling of the body core temperature was greater ($p < 0.05$) in iron deficiency than after iron repletion and supplementation (-0.43 ± 0.04 and -0.14 ± 0.02 $°C \cdot h^{-1}$, respectively). Similarly, iron deficiency was associated with an increased ($p < 0.05$) cooling of the periphery (-3.4 ± 0.1 vs -2.4 ± 0.1 $°C \cdot h^{-1}$).

Pre-exposure values of oxygen consumption and metabolic heat production were unaffected ($p > 0.05$) by dietary treatment (Fig. 2). Both variables increased ($p < 0.05$) during cold exposure. The oxygen consumption and metabolic heat production were less ($p < 0.05$) during iron depletion than after iron repletion. The increase in oxygen uptake was less ($p < 0.05$) in iron depletion ($\dot{V}O_2 = 3.43 + 0.004t$, $r = 0.98$ vs. $\dot{V}O_2 = 3.54 + 0.006t$, $r = 0.98$). The rate of oxygen utilization was depressed ($p < 0.05$) during iron deficiency (37.8 ± 4.2 vs. 62.6 ± 3.6 $ml \cdot h^{-1}$). Similarly, the increase in metabolic rate was less ($p < 0.05$) with iron deficiency ($M = 45.4 + 0.057t$, $r = 0.98$ vs. $M \pm 46.2 + 0.074t$, $r = 0.98$). The relative change from pre-exposure values of oxygen uptake and metabolic rate in the cold was less ($p < 0.05$) with iron depletion than repletion and supplementation (9 ± 1 vs. $14.5 \pm 1.5\%$).

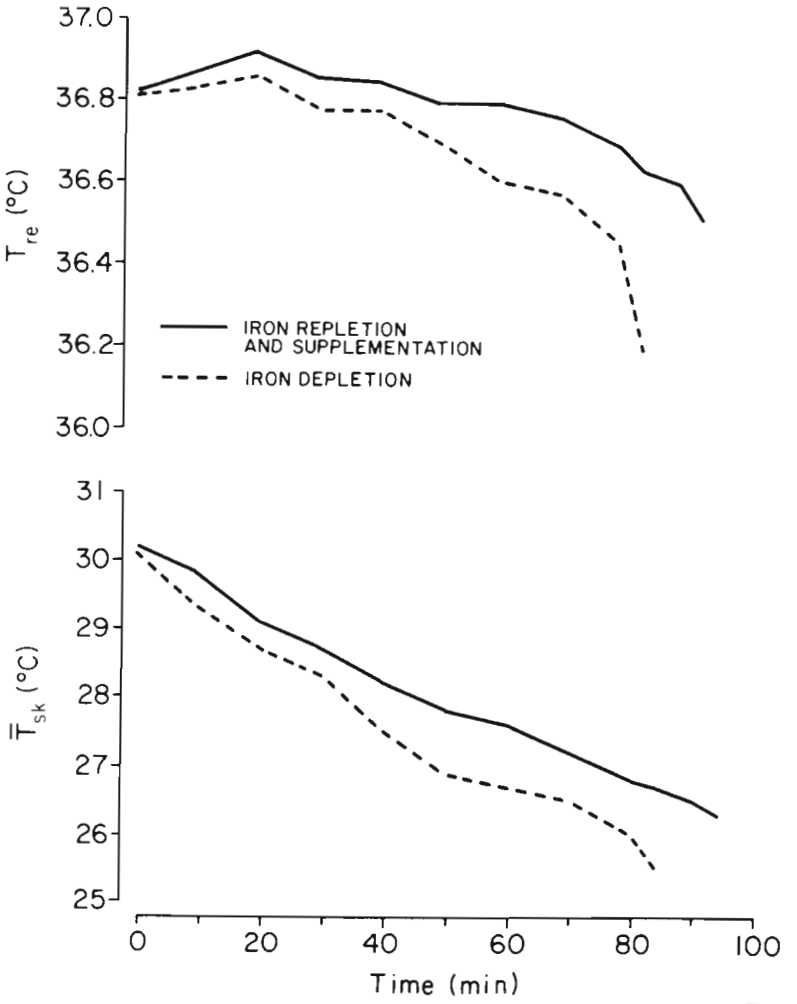

Fig. 1. Mean rectal (T_{re}) and the average of mean skin ($\bar{T}_{sk}$) temperatures of 11 women during cold exposures at 16°C after iron deficiency and iron repletion and supplementation. The S.E. for each T_{re} was ± 0.1°C and the S.E. for each $\bar{T}_{sk}$ was ± 0.2°C.

The relationships between core temperature and oxygen consumption relative to iron status are presented in Fig. 3. During iron deficiency, the slope and intercept were each significantly ($p < 0.05$) less ($\dot{V}O_2 = 22.1–0.50$ T_{re}, $r = -0.88$ vs. $\dot{V}O_2 = 51.9–1.31$ T_{re}, $r = -0.87$) than that observed after iron repletion and supplementation. The shift of this relationship downward and to the left during iron deficiency suggests a change in the core temperature for the onset of shivering.

The iron-deficient women ($n = 11$) had a depressed ($p < 0.05$) average rate of oxygen utilization (Table III)

TABLE II. BLOOD BIOCHEMICAL INDICES OF IRON STATUS (N = 11)*

	Entry	Depletion	Repletion and Supplementation
Hemoglobin, $g \cdot dl^{-1}$	13.4 ± 0.2^a	12.0 ± 0.2^b	12.6 ± 0.1^a
Hematocrit, %	38.5 ± 0.6^a	35.5 ± 0.4^6	36.4 ± 0.4^a
Ferritin, $ng \cdot ml^{-1}$	26.3 ± 5.1^a	5.5 ± 0.5^b	9.5 ± 0.4^c

* Values are mean ± S.E.
[a,b,c] Values with different superscripts in same row are statistically different ($p < 0.05$).

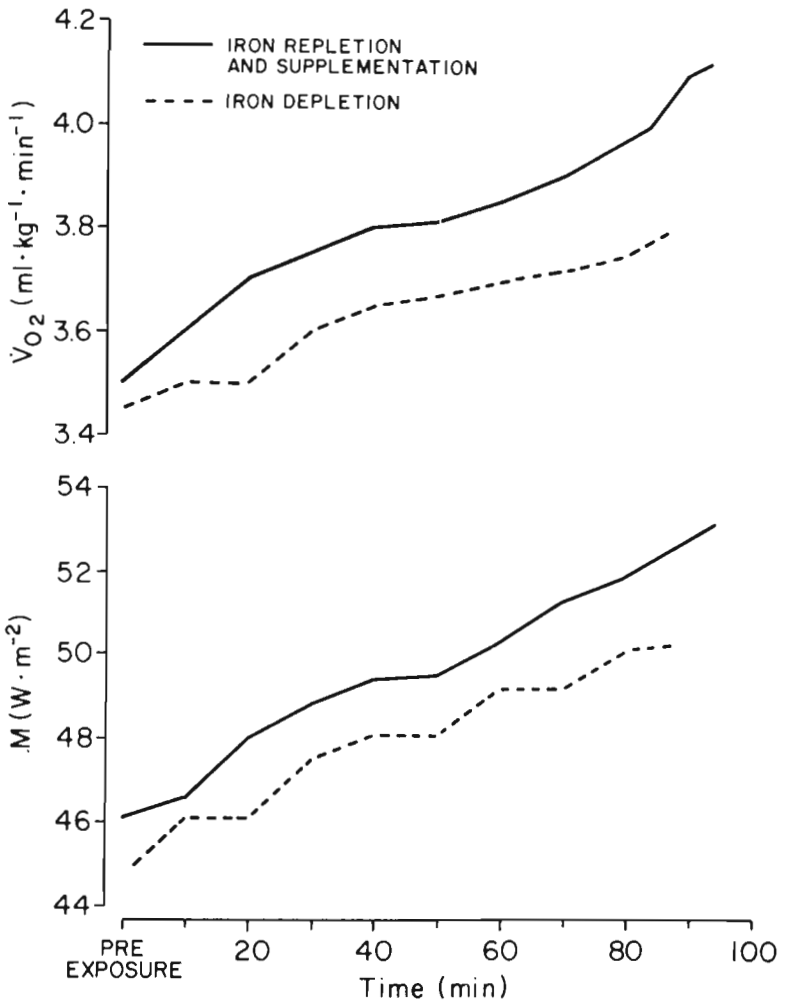

Fig. 2. Mean oxygen consumption ($\dot{V}O_2$) and mean metabolic rate (M) of 11 women during cold exposures at 16°C after iron deficiency and iron repletion plus supplementation. The S.E. for each $\dot{V}O_2$ was ± 0.1 ml · kg$_{-1}$ · min^{-1} and for each M was ± 1.0 W · m^{-2}.

and a 10% reduction in total oxygen uptake (19.9 ± 0.6 vs. 22.1 ± 0.5 L; $p < 0.05$). Iron deficiency elevated ($p < 0.05$) the mean respiratory exchange ratio and reduced ($p < 0.05$) metabolic heat production. Core temperature, mean skin temperature and mean body temperature declined more ($p < 0.05$), and the body heat debt increased more after iron deficiency than after iron repletion and supplementation.

Supplementation with nutrients other than iron appeared to exert no demonstrable effect on thermoregulatory responses (Table III). No significant effect of calcium supplementation during iron depletion was found on physiological responses during cold exposure. Similarly, ascorbic acid supplementation during iron repletion and supplementation did not appear to influence physiological responses during the cold exposures.

Because of difficulties in either inserting venous catheters or maintaining them patent, pre- and post-exposure blood specimens were obtained in only six women (Table IV). Circulating thyroid-stimulating hormone (TSH) and thyroid hormone concentrations increased after cold exposure. The increase ranged from 16–18% during iron deficiency and from 22–25% after repletion and supplementation. There was no statistical ($p > 0.05$) difference between TSH or thyroid hormone concentrations relative to iron status in these six women before and after cold exposure. Catecholamine concentrations increased ($p < 0.05$) after cold exposure. Epinephrine concentrations were not different during iron depletion and repletion. Norepinephrine concentrations were greater ($p < 0.05$) after cold exposure during iron depletion than after iron repletion and supplementation.

No statistical differences were found in hormonal responses relative to calcium or ascorbic acid supplementation by dietary treatment (Table IV).

DISCUSSION

The findings of the present study suggest that iron deficiency without anemia is associated with an earlier onset of visible shivering in women acutely exposed to cold and is due to impaired non-shivering thermogenesis. Some factors are useful in explaining this observation.

Iron deficiency, characterized by a significant negative iron balance, resulted in an increased rate of cooling of body core temperature. The decrease in T_{re} was significantly greater during iron depletion. Concomitantly, $\overline{T}_{sk}$ was depressed; it declined at a faster rate in iron deficiency. These observations suggest that neural input from central and peripheral areas indicated a need for increased heat production that was not achieved with an appropriately increased oxygen uptake during iron deficiency.

Another explanation is a change or downward shift in the core temperature threshold for shivering. Although this cannot be documented unequivocally in the present study because volunteers were removed from the cold before prolonged shivering, data presented in Fig. 3 indicate a blunted oxygen uptake response relative to the faster rate of core cooling in iron deficiency. Such an impaired response in metabolic heat production is consistent with a lower core temperature threshold for the onset of shivering, particularly when pre-exposure oxygen uptake is similar among the iron depleted and repleted women. Thus, the increased rate of core cooling and the inability to increase metabolic heat production result in a reduced core temperature threshold for shivering.

Our finding of a reduced core temperature during cold exposure in iron deficiency is consistent with the cross-sectional observations of Martinez-Torres, *et al.* (24), who reported that oral temperatures fell −0.9, −0.5, and −0.2°C in anemic, iron-deficient, and iron-adequate subjects, respectively, after immersion in 28°C water for 1 h. Oxygen uptake, however, during cold exposure was significantly greater in anemic and iron-deficient than iron-adequate individuals. The authors attribute this elevated oxygen consumption to an apparent increase in heat loss associated with impaired iron status.

Our findings of decreased oxygen uptake during cold exposure in iron deficiency conflicts with the observations of Martinez-Torres, *et al.* (24). These conflicting findings may be reconciled on the basis of body composition. In the study of Martinez-Torres, *et al.* (24), body composition was neither determined nor controlled in the three groups of participants. Height to weight ratios were reported as 2.61, 2.66, and 3.03 cm · kg^{-1} for control, iron-deficient, and anemic subjects, respectively. The ratio is greater ($p < 0.05$) for the anemic individuals, suggesting that differences in body composition might be present among the groups. In the

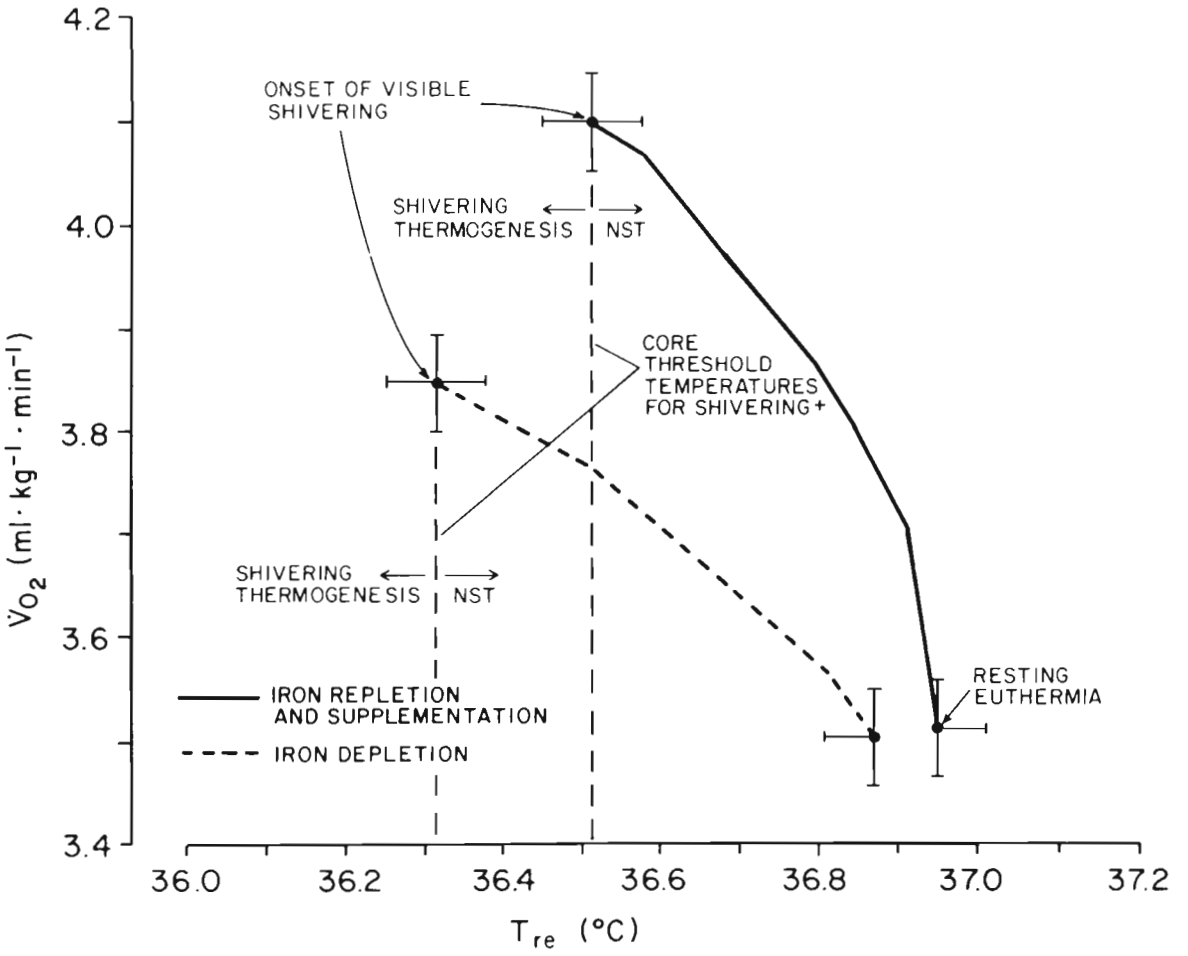

Fig. 3. Relationship between mean oxygen uptake ($\dot{V}O_2$) and mean rectal temperature (T_{re}) of 11 women during acute cold exposure during iron deficiency and after iron repletion and supplementation. The S.E. for the relationship during depletion was ± 0.06 and after repletion was ± 0.10 ml · kg^{-1} · min^{-1}. NST is nonshivering thermogenesis. +Shivering was observed visually.

TABLE III. PHYSIOLOGICAL RESPONSES DURING COLD EXPOSURE*

	Depletion			Repletion and Supplementation		
	"−"Ca† (n = 5)	+Ca (n = 6)	Total (n = 11)	"−"AA (n = 6)	+AA (n = 5)	Total (n = 11)
$\dot{V}O_2$††, ml · kg^{-1} · min^{-1}	3.6 ± 0.1	3.5 ± 0.1	3.6 ± 0.1§	4.0 ± 0.1	3.8 ± 0.1	3.9 ± 0.1
M, W · m^{-2}	50.1 ± 1.0	49.1 ± 1.2	49.6 ± 1.1§	53.2 ± 1.4	54.0 ± 1.3	53.6 ± 1.2
RER	0.78 ± 0.03	0.83 ± 0.04	0.80 ± 0.03§	0.78 ± 0.02	0.76 ± 0.03	0.74 ± 0.02
ΔT_{re}, °C	−0.5 ± 0.1	−0.7 ± 0.2	−0.6 ± 0.1§	−0.2 ± 0.1	−0.2 ± 0.1	−0.2 ± 0.1
$\Delta \bar{T}_{sk}$, °C	−4.5 ± 0.3	−4.5 ± 0.3	−4.5 ± 0.2§	−3.4 ± 0.1	−3.2 ± 0.1	−3.3 ± 0.2
ΔT_b, °C	−1.8 ± 0.2	−1.9 ± 0.2	−1.9 ± 0.1§	−1.3 ± 0.1	−1.2 ± 0.1	−1.2 ± 0.1
S, W	37.2 ± 1.3	35.8 ± 1.1	36.3 ± 1.1§	25.2 ± 1.0	24.4 ± 1.2	24.7 ± 1.1

Values are mean ± S.E.
† Refers to the ues of calcium (Ca; 800 mg · d^{-1}) and ascorbic acid (AA; 1,500 mg · d^{-1}) supplements to the diet; "−" refers to the use of a placebo.
†† $\dot{V}O_2$ = oxygen uptake; M = metabolic heat production; RER = respiratory exchange ratio; ΔT_{re} = change in core temperature; $\Delta \bar{T}_{sk}$ = change in mean skin temperature; ΔT_b = change in mean body temperature; S = body heat debt.
§ Significantly ($p < 0.05$) different than value determined after repletion and supplementation (n = 11).

present longitudinal study, percent body fat, sum of skinfolds, and fat-free mass were unchanged for each cold test. The importance of controlling body composition, and particularly percent body fat, in determining the metabolic response of humans to cold exposure is well established (6).

Short term increases in metabolic heat production to maintain body temperature are dependent upon thyroid hormones and catecholamines (16). At room temperature (23–24°C), we observed no differences in TSH and thyroid hormone concentrations relative to iron status. After cold exposure, the plasma concentrations of these hormones increased. It is noteworthy that the relative increases in TSH, T_4, and T_3 were smaller (18, 16, and 18%, respectively) when iron balance was negative than when it was positive (23, 23, and 25%, respectively). These findings of a blunted increase in circulating thyroid hormone concentrations during cold exposure in iron-deficient humans are reasonably consistent with a previous report of impaired thyroid hormone metablism in anemic rats exposed to cold (11). Some recent data from Beard, *et al.* (4), suggest that anemia, rather than tissue iron depletion, though not measured, may be responsible for the impaired production of T_3. Importantly, they also showed a blunted increase in TSH, T_4, and T_3 in anemic rats exposed to cold after transfusion of erythrocytes to increase hematocrit to near normal values. A similar observation in the present human study parallels these findings and indicates that depleted body iron stores (e.g., negative iron balance) influence thyroid hormone metabolism.

Sympathetic nervous system activity in humans and

TABLE IV. HORMONAL RESPONSES TO COLD EXPOSURE IN 6 WOMEN*

		Depletion			Repletion and Supplementation		
		"−"Ca† n = 3	+Ca n = 3	Total n = 6	"−"AA n = 3	+AA n = 3	Total n = 6
TSH††, $\mu IU \cdot ml^{-1}$	REST	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.3
	COLD	1.3 ± 0.2	1.3 ± 0.1	1.3 ± 0.2	1.5 ± 0.2	1.6 ± 0.3	1.6 ± 0.3
T_4, $ng \cdot dl^{-1}$	REST	1.2 ± 0.2	1.2 ± 0.1	1.2 ± 0.2	1.3 ± 0.1	1.3 ± 0.2	1.3 ± 0.1
	COLD	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
T_3, $ng \cdot ml^{-1}$	REST	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2
	COLD	1.3 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.2	1.6 ± 0.1	1.5 ± 0.1
Epinephrine, $pg \cdot ml^{-1}$	REST	64 ± 6	61 ± 7	63 ± 7	70 ± 8	75 ± 7	72 ± 9
	COLD	215 ± 15#	222 ± 14#	218 ± 16#	209 ± 7#	198 ± 8#	201 ± 9#
Norepinephrine, $pg \cdot ml^{-1}$	REST	127 ± 18	136 ± 14	131 ± 17	163 ± 15	159 ± 16	160 ± 19
	COLD	1160 ± 15#	1165 ± 11#	1162 ± 12#§	751 ± 17#	734 ± 12#	740 ± 14#

* Values are mean ± S.E.
† Refers to the use of calcium (Ca; 800 $mg \cdot d^{-1}$) and ascorbic acid (AA; 1500 $mg \cdot d^{-1}$) supplements to the diet; "−" refers to the use of a placebo.
†† TSH = Thyroid stimulating hormone; T_4 = thyroxine; T_3 = triiodothyronine.
# Significantly ($p < 0.05$) different than rest value.
§ Significantly ($p < 0.05$) different than value determined after repletion and supplementation (n = 6).

animals increases during cold exposure. The increase is characterized by elevated circulating concentrations and increased urinary excretion of norepinephrine (16). When acutely exposed to cold, anemic rats and humans have significantly elevated plasma norepinephrine and excrete more norepinephrine than nonanemic controls (10,32,33). This abnormality in the cold-stressed animals is independent of anemia (10).

In the present study, we observed similar pre-exposure plasma norepinephrine concentrations among iron depleted and repleted women. After cold exposure, a greater increase in plasma norepinephrine concentrations was observed during iron deficiency than after iron repletion and supplemention. In parallel with the elevated plasma norepinephrine, mean skin temperatures were significantly lower during iron deficiency. The elevated norepinephrine concentrations are likely the result of enhanced sympathetic activity to cause vasoconstriction in peripheral tissues of the limbs and thus to reduce heat loss through the skin. These factors indicate a primary mechanism of response during iron deficiency to cold exposure. This observation is consistent with findings in which the capacity for peripheral vasoconstriction appeared normal in iron-deficient animals (10).

Two additional explanations are also available. Based upon the results of animal studies, it can be argued that increased blood norepinephrine concentration may be a compensatory response for the maintenance of body temperature caused by the blunted thyroid hormone response (10). Dallman (8) suggested that the increased norepinephrine was the result of a decrease in norepinephrine degradation cause by a reduced activity of monoamine oxidase, an iron-dependent enzyme. Beard (3) demonstrated high rates of norepinephrine turnover and a limited catabolic capacity for it in iron-deficient animals.

In contrast to some reports in the literature, we found no significant effect of supplemental ascorbic acid or calcium on human metabolic or physiological responses during cold exposure. Differences in experimental procedures and design may explain the discrepancies. The studies (13,22) showing that calcium affects the set point control of body temperature utilized cats receiving direct injections in the posterior hypothalamus of fluids containing calcium at concentrations exceeding the physiological range by 14–68 mM. The beneficial affect of ascorbic acid supplementation (325 $mg \cdot d^{-1}$) on reducing the fall in core and muscle temperatures of monkeys exposed to −20°C cold required a 6-month dietary equilibration period (12). In contrast to these animal experiments, nutrients were neither injected nor were prolonged equilibration periods used in the present human study. It is unclear at present whether oral pharmacologic doses of calcium administered to humans can actually influence temperature regulatory function. Also, the significance of oral ascorbic acid supplementation on human physiological function in the cold remains unknown.

In the present study, we anticipated greater increases in serum ferritin concentration after iron repletion using dietary iron and oral iron supplements than we observed. Jacobs, *et al.* (18), previously reported a similar blunted response in serum ferritin in men after repeated phlebotomy and observed a significant increase in hemoglobin from about 10.5 to 12.5 $g \cdot dl^{-1}$. Our finding in the present study parallels their observation that serum ferritin may be an unreliable indicator of body iron stores among individuals undergoing phlebotomy.

In summary, iron-deficient, but not anemic, humans respond to acute cold exposure with impaired, nonshivering thermogenesis and increased body heat debt. This response is associated with a rapid cooling of body core temperature and depressed mean skin temperature. The net effect is to shift the lower critical temperature for shivering. This appears to be an adaptation in thermoregulatory function for cold tolerance. The hormonal response to acute cold exposure in iron-deficient humans includes a blunted increase in thyroid hormones and a marked increase in circulating norepinephrine. The exaggerated norepinephrine response appears necessary to attempt to compensate for the reduced ther-

mogenesis and to increase vasoconstriction to reduce surface heat loss and thus to preserve deep body temperature.

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